PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference Case 21421	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/10403	International filing date *(day/month/year)* 18.09.2003	Priority date *(day/month/year)* 27.09.2002
International Patent Classification (IPC) or both national classification and IPC C12P17/12		
Applicant DSM IP ASSETS B.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
11.03.2004	21.01.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Schneider, P Telephone No. +31 70 340-4523

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/10403

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-10 as originally filed

Claims, Numbers

1-6 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority in written form.

   ☒ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-6
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-6
Industrial applicability (IA)	Yes:	Claims	1-6
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The following documents (D) are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1: YANG YONG ET AL JOURNAL OF BACTERIOLOGY, vol. 180, no. 16, August 1998 (1998-08), pages 4294-4299
D2: EP-A-0 950 715 (HOFFMANN LA ROCHE) 20 October 1999 (1999-10-20)

1 Novelty (Art. 33(2) PCT)

The present application discloses recombinant microorgansims and processes for the production of vitamin B_6 through the introduction of extra genes encoding:
i) erythrose 4-phosphate dehydrogenase (Epd) alone or
ii) Epd and 1-deoxy-D-xylulose-5-phosphate synthase (Dxs) or
iii) Epd and pyridoxol 5'-phosphate synthase (PdxJ) or
iiii) Epd and Dxs and PdxJ.
No prior art document discloses the introduction of extra gene copies of said genes involved in the vitamin B_6 biosynthesis pathway. Therefore, novelty under Art. 33(2) PCT is given.

2 Inventive Step (Art. 33(3) PCT)

2.1 Document D2 is the closest prior art and discloses the production of vitamin B_6 using a cell free extract of i.a. E.coli from 1-deoxy-D-threo-pentulose (DTP) and 4-hydroxy-L-threonine (HT) using the same standard culture conditions as the present application (see [0001] to [0003] and [0007]).
From this the subject matter of the present application differs in that an in vivo method of production of vitamin B_6 using i.a. E.coli having extra genes as mentioned under point 1 is used.
The only technical effect that can be seen to be associated with said difference is the use

of an in vivo method without the necessity to add educts and cofactors (like DTP, HT, NAD^+ and ATP).
The technical problem to be solved is therefore the provision of an alternative, simplified method to produce vitamin B_6 (see also applicants reply dated 05.10.2004).

2.2 It is well known in the art that vitamin B_6 is an essential vitamin which is not biosynthetically produced by e.g. human. It is an important medicine and food additive for humans (see D2, p.2, [0002] and [0003]). There, it is also described that there is an interest in the fermentative production of vitamin B_6, although no commercially attractive fermentation process was known so far. There was a general incentive to provide any sort of improved, simplified alternative production method. Therefore, the technical problem to be solved was already known in the art.

2.3 From his common general knowledge the skilled person is aware of the general fact that if the enhanced production of the end product of a biosynthetic pathway is desired, one efficient way to do so would be to enlarge the number of any of the enzyme molecules catalysing the biosynthetic steps on this pathway or a combination thereof. The fact that D2 describes that there is an interest in the fermentative production of vitamin B_6, although no commercially attractive fermentation process was known so far ([0003]) does not constitute a prejudice in the art as the cited documents in D2 [0003] are too old to take into account the possibilities of recombinant DNA technologies, which, by contrast, are well known to the skilled person working in this field at the time of filing.
As a consequence, the skilled person looking for a solution to the above-mentioned problem to be solved **would** clearly consider D1 as it discloses the complete vitamin B_6 biosynthesis pathway with the enzymes involved in E.coli starting from D-erythrose-4-phosphate. It would be apparent and obvious to him to try to create an vitamin B_6 overproducer strain by overexpressing one or more of the biosynthetic genes disclosed in D1. All the necessary methods to do so (cloning and expressing known enzymes in its natural host) are well established in the art.
As a consequence the skilled person would introduce extra copies of any of the involved enzyme alone or in combination and investigate which combination gives the best result in view of vitamin B_6 production. In other words, he would arrive at the solution presented in independant claims 1 and 4 of the present application with a reasonable expectation of success by applying standard knowledge and techniques, i.e. without exercising inventive skills.

The skilled person might also envisage the measures mentioned in the last but one paragraph of the reply but the creation of a recombinant overproducer appears more promising and easier to use once created. These mentioned measures make even more sense using such a recombinant overproducer strain.

2.4 If the introduction of a combination of extra genes is envisaged, the skilled person would expect an exponential enlargement of the amount of end product produced due to the catalytic action of enzymes. Therefore, the factors of increase of vitamin B_6 shown in Table 1 (p.9/10) of the present application are not surprising and therefore not suitable to establish an inventive step.
The subject matter of any dependant claim consists of standard techniques not suitable to establish an inventive step. As a consequence, in the absence of a hitherto unknown or surprising technical effect, which cannot be seen in the application as filed, no claim appears to fulfill the requirements of Art. 33(3) PCT.

3 Apart from the above-mentioned novelty and inventive step objections the present claims fulfill the requirement of industrial applicability (Art. 33(4) PCT).

4 Unity (Rule 13.1 PCT)

As a consequence of the objection raised under point 2, the common concept linking together the four different solutions of the present application (see point 1, i) to iiii)) is not inventive and they form four different inventions.